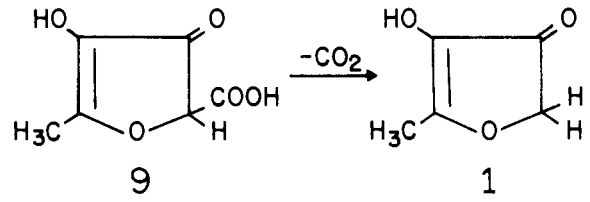

Figure 3.

rived Amadori compounds could give rise to **9** (Figure 3) which is a β-keto acid and would be expected to readily decarboxylate to the furanone (**1**) incorporating one solvent deuterium atom in the process.

LITERATURE CITED

Anet, E. F. L. J., *Adv. Carbohydr. Chem.* **19,** 181 (1964).
Bray, G. A., *Anal. Biochem.* **1,** 279 (1960).
Feather, M. S., Harris, J. F., *J. Org. Chem.* **31,** 4018 (1966).
Feather, M. S., Harris, J. F., *Adv. Carbohydr. Chem.* **28,** 161 (1973).
Heyns, K., Baltes, W., *Chem. Ber.* **93,** 1616 (1960).
Hicks, K. B., Harris, D. W., Feather, M. S., Loeppky, R. N., *J. Agric. Food Chem.* **22,** 724 (1974).
Hodge, J. E., Fisher, B. E., Nelson, E. C., *Proc., Am. Soc. Brew. Chem.,* 84 (1963).
Hodge, J. E., Mills, F. D., Fisher, B. C., *Cereal Sci. Today* **17,** 34 (1972).
Hodge, J. E., Nelson, E. C., *Cereal Chem.* **38,** 207 (1961).
Maciak, G., *Methods Carbohydr. Chem.* **1,** 461 (1962).
Marsh, C. A., *J. Chem. Soc.,* 1578 (1952).
Micheel, F., Hagemann, G., *Chem. Ber.* **92,** 2836 (1959).
Peer, H. G., Van Den Ouweland, G. A. M., DeGroot, C. N., *Recl. Trav. Chim. Pays-Bas* **87,** 1011 (1968a).
Peer, H. G., Van Den Ouweland, G. A. M., DeGroot, C. N., *Recl. Trav. Chim. Pays-Bas* **87,** 1017 (1968b).
Schmidt, O. T., *Methods Carbohydr. Chem.* **2,** 322 (1963).
Severin, Th., Seilmeier, W., *Z. Lebensm.-Unters.-Forsch.* **137,** 4 (1968).
Tonsbeek, C. H. T., Plancken, A. J., Weerdhof, T. v. d., *J. Agric. Food Chem.* **16,** 1016 (1968).

Received for review November 15, 1974. Accepted May 12, 1975. Journal paper No. 7146 of the Missouri Agricultural Experiment Station. We thank the Corn Refiners Association and the National Science Foundation (Grant No. GP-38511) for financial support of this project.

Melanoidins and Carbohydrates in Roasted Barley

B. Lj. Milić,* B. Grujić-Injac, M. V. Piletić, S. Lajšić, and Lj. A. Kolarov

Carbohydrate constituents of barley have been determined. Changes occurring during the process of roasting, mainly the interaction between carbohydrates and amino compounds, have been studied. A brown amorphous substance of melanoidin type was isolated by column chromatography on ion-exchange resin Permutit ES. Its simplest empirical formula of $C_{18}H_{27}O_{11}N$ was determined by elementary analysis which was compared to a model substance obtained from reaction between L-aspartic acid and D(+)-glucose at 93° for 230 hr. The empirical formula of the melanoidin model substance was found to be $C_{18}H_{26}O_{11}N$. Both ir and uv spectra of melanoidins isolated from roasted barley and model substance were found to be similar. A method for determination of melanoidins by spectrophotometry at 430 nm was developed.

The changes of constituents during the roasting of cereals have been largely concerned with the changes in concentration of nonstructural carbohydrates, which amounts to over 60% in unroasted grain. Underwood and Deatherage (1952) found that the concentration of water-soluble constituents, primarily nonstructural carbohydrates, decreases during the process of roasting. On the other hand, some simple constituents are formed as shown by a positive test for carbohydrates of glucose and saccharose type. The monosaccharides were changed during the process of roasting so that one part, in reaction with proteins and amino acids, produced complexes of melanoidin and the other part was lost by decarboxylation and dehydration. Wolfrom et al. (1960) reported that holo- and hemicellulose have been slightly decreased during the roasting of coffee beans. Knauf et al. (1941) reported that anhydromannose in defatted palm seeds was degraded into a soluble form of 1,6-anhydro-α-D-mannopyranose by pyrolysis during the process of roasting.

Roasting of cereals at a temperature of 200° is inevitably followed by the formation of brown pigments of melanoidin type (Holtermand, 1966; Reynolds et al., 1962; Anet, 1960, 1961, 1962; Burton and McWeeny, 1964). Wolfrom (1945) found that the sugar-C:N:methylene-C ratio must be 1:1:1 for the optimal production of melanoidins while Anet (1961, 1962) has shown that the ratio of sugar-C:N may be 6:1. This confirms that melanoidins can be of different compositions from various sources (Grujić-Injac et al., 1971). Kass and Palmers (1940) state that the formation of brown pigments at high temperatures does not allow the interpretation of the "interaction" between amino acids and reducing sugars as a bifunctional reaction. In fact, this "interaction" is complex and unidentified. Formation of melanoidins is due to caramelization of sugar and adsorption of aldocaramel on colloidal particles of protein.

In the preparation and processing of foods, one is soon acquainted with the phenomenon of browning associated with heated and stored products. These reactions do not require enzymic catalysis and are referred to as nonenzymic. Many food industries are directly concerned with the production of these brown products, to the extent that they contribute to the flavor, color, and aroma of their products, e.g., coffee, caramel, bread, and breakfast cereals. However, even in these processes careful control must be exerted to prevent excessive browning, which could lead to unpleasant changes occurring in the food product.

This study was undertaken to investigate the influence of applied temperature on food processing. It was interesting to study both the concentrations and changes of carbohydrates and amino acids during the roasting of barley

Faculty of Technology, Novi Sad University, Novi Sad, Yugoslavia (B.L.M., M.V.P., L.A.K.), and Faculty of Sciences, Belgrade University, Belgrade, Yugoslavia (B.G.-I., S.L.).

and explain the nature of their interaction and formation of the melanoidins.

EXPERIMENTAL SECTION

Materials and Methods. Barley was used as the experimental material to study the influence of roasting on changes in carbohydrates and amino acids. Two samples of barley, from 0.7 to 1.0 kg, were used. They were obtained from the industrial production of roasted barley after 10, 20, 30, 40, and 50 min of roasting at a temperature of 250°. The roaster was a Type SAS Infrator Scolary (Milan, Italy).

Determination of Total Sugar. The barley was ground in a Condux mill to pass an 80-mesh screen. A 2.5-g sample was extracted with 100 ml of cold water with constant shaking for 4 hr. After extraction, 2.5 g of Celite 545 was added to the suspension to aid filtration. The filtrate was brought to 100-ml volume with distilled water. A 50-ml aliquot was acidified with 5 ml of 0.1 *N* H_2SO_4 and heated to coagulate proteins, which were removed by filtering the cooled solution through the layer of Celite 545 and filter paper in a Büchner funnel. Then 15 ml of the clear filtrate was transferred into the thin-walled test tube with the ground neck and 5 ml of H_2SO_4 (2.0 *N*) was added. The tube was attached to a condenser and the solution was refluxed for 20 min in a boiling water bath. After cooling, the hydrolysate was neutralized with 2.0 *N* NaOH and the solution was diluted with water to 25 ml. The solution was then passed through an activated ion-exchange resin column (7.0 × 1.0 cm) of Lewatit MIH and Lewatit PN. The deionized solution (5.0 ml) was analyzed for the reducing sugars by the method of Wiseman et al. (1960).

Reducing sugars, starch, hemicellulose, and cellulose were determined on separate samples, prepared in a similar way, according to the method of Milić and Vlahović (1971). Dextrin and α-amino nitrogen were determined in the original sample by AOAC Official Methods (1960).

Brown Pigments; Melanoidins. Brown pigments of the melanoidin type were determined using a model system as the standard substance. The standard substance was obtained by the reaction of 0.5 *M* glucose and 0.25 *M* L-aspar tic acid at pH 2.3 at a temperature of 93°. Precipitation of the brown amorphous substance was initiated after the reaction mixture was kept under the above conditions for 112 hr. Precipitation of the polymerization product was discontinued after 230 hr. Examination of the reaction mixture by paper chromatography before and after the described reaction revealed that several substances were formed. L-Aspartic acid and glucose were identified prior to the reaction, while L-aspartic acid, alanine (traces), glycine (traces), and acetyl aldehyde (traces) were identified after the reaction period. After 230 hr the reaction mixture had the characteristic odor of roasting.

The brown amorphous substance was purified on a column of ion-exchange resin Permutit ES (2.5 × 35 cm). The resin was converted into the $(OH)^-$ form by passing 480 ml of 5% aqueous NaOH solution through the column. It was then washed with excess alkali and converted into the $(Cl)^-$ form by treatment with 420 ml of 5% aqueous NaCl solution. The resin was cleared of excess chloride by rewashing with deionized water.

The amorphous brown substance of the model system, obtained from reaction of L-aspartic acid and glucose, and by evaporation of the reaction solution at 50° under reduced pressure to dryness, was dissolved in a small portion of water and applied to a column of activated Permutit ES. Interfering substances of the nonmelanoidin type were eluted from the column with 2000 ml of deionized water. The melanoidins were eluted with 5% NaCl solution until the brown color in the effluent completely disappeared. The effluent was concentrated by evaporation at 45° under high vacuum. The $(Cl)^-$ ions were removed by dialyzing and the solution was reevaporated to dryness under the same conditions and dried over P_2O_5 at 40° under high vacuum for 48 hr.

Table I. Percentages of Some Carbohydrates, Melanoidins, and α-Amino Nitrogen in Roasted Barley

Constituents	Time of roasting, min					
	0	10	20	30	40	50
Total sugars, %	3.49	3.75	4.28	4.53	5.75	6.25
Reducing sugars, %	2.32	2.58	3.75	3.98	4.38	3.05
Starch, %	55.75	54.85	53.11	52.56	50.73	51.25
Dextrin, %	0.48	0.96	0.98	1.75	3.38	4.38
Hemicellulose, %	3.36	3.30	3.30	3.05	2.01	1.13
Cellulose, %	4.82	4.96	4.75	4.60	4.66	4.72
Melanoidins, %		0.35	0.85	1.12	3.57	4.15
α-Amino N, mg %	18.48	15.39	12.20	7.79	5.20	14.67

The weighed dry substance (0.1 g) was dissolved in 250 ml of water and a series of graded concentrations was prepared based on color intensity determined on a spectrophotometer at a wavelength of 430 nm. A calibrated curve was constructed from these results.

Samples of barley were ground to pass an 80-mesh screen and were extracted with 250 ml of deionized water with mechanical shaking for 4 hr. The extracts were allowed to pass through an activated column of Permutit ES. Purification was made in the same way as for the model system. Color intensity was measured at 430 nm and the values were calculated according to the calibrated curve of the model system.

Uv spectra of both the isolate and the model system were measured at wavelengths from 190 to 370 nm on the Unicam Spectrophotometer, Model SP 800. Ir spectra were determined on the Unicam IR spectrophotometer, Model SP 1200.

Melanoidins isolated from barley and from the model system were analyzed for the percentage of C, H, N, and O using Elemental Analyzer Carlo Erba, Model 1102, by gas chromatography. Its detector and recorder were connected to a Digital Integrator Vidar, Model 6300-2, for automatic calculation of separated peaks. The percentages of amino acids of both unroasted and roasted samples of barley were analyzed in the hydrolysates by a Bio-Cal amino acid analyzer, Model BC-200.

RESULTS AND DISCUSSION

Concentrations of carbohydrates and melanoidins in samples of unroasted and roasted barley are presented in Table I. As shown in Table I, a significant increase in total sugars was observed during the process of roasting from 3.49%, for the original unroasted sample, to 6.25% for the sample of barley roasted for 50 min. The increase of reducing sugars from 2.32%, for the original, to 4.38% for the sample roasted for 40 min can be explained by the decrease in starch from 55.75% in the original sample to 51.25% in the sample of barley roasted for 50 min. Hemicellulose underwent degradation under the influence of temperature. A decrease of about 55% during the process of roasting for 50 min is very significant. An increase of dextrin from 0.48% in the original sample to 4.38% in the sample roasted for 50 min was expected, and is in agreement with loss of starch in the same sample. α-Amino nitrogen in the original sample amounted to 18.48 mg %, while that value decreased to 5.20

Table II. Changes in Amino Acids of Barley during the Process of Roasting (% d.w.)

Amino acid	Time of roasting, min		
	0	20	30
Lys	0.28	0.20	0.16
His	0.18	0.10	0.08
Arg	0.45	0.15	0.08
NH_3	0.22	0.15	0.11
Asp	0.46	0.32	0.12
Thr	0.27	0.21	0.14
Ser	0.31	0.17	0.08
Pro	0.80	0.81	0.81
Glu	1.96	2.11	2.44
Gly	0.31	0.38	0.35
Ala	0.32	0.36	0.38
Val	0.44	0.45	0.44
Met	0.14	0.10	0.05
Ile	0.31	0.30	0.30
Leu	0.56	0.50	0.48
Tyr	0.34	0.28	0.21
Phe	0.40	0.40	0.41

mg % in the sample roasted for 40 min. In barley roasted to near carbonization at a temperature of 250° for 50 min α-amino nitrogen increased to 14.67 mg % as compared with 5.20 mg % at 40 min, which is certainly due to protein denaturation.

The brown pigment of melanoidin type, approximately determined from the model system, was found only in traces in the original sample. However, during the process of roasting, its amount increased to a maximum value of 4.15% after 50 min.

The percentage of amino acids in unroasted and roasted samples of barley and their changes during the process of roasting are presented in Table II. The percentages of all basic amino acids (histidine, lysine, and arginine) and ammonia were lower in the samples of barley which were roasted for 20 and 30 min. The results shown in Table II show a significant decrease of some neutral amino acids (threonine, serine, methionine, leucine, and tyrosine) and one acidic amino acid (aspartic acid). The higher concentration of glutamic acid, glycine, and alanine was found in the sample of roasted barley, which was unexpected, especially for glycine and alanine as the most reactive amino acids. The obtained data, shown in Table II, were in good accordance with those found by Wasserman and Spinelli (1970) in the samples of heated meat–water extracts. The percentage of the other amino acids remained nearly unchanged during the process of roasting. The changes, which happened during the roasting of barley and which referred to the decrease of some amino acids, were due to the denaturation of proteins under the influence of temperature and to the reaction of amino acids with carbonyl compounds. Lea and Hannan (1950) reported that the decrease in free α-amino nitrogen and amino acids is the result of an interaction of amino compounds and sugars, which occurred during the process of concentration and storage of liver extract.

It is known that sulfur amino acid or lysine is the limiting nutritional factor of most protein foods and diets. In some of these foods, either before or after processing, a proportion of these amino acids has been shown to be unavailable. Maillard reactions and other C–N cross-links occurring during severe heat processing of food are known examples of these phenomena. The losses occurring during the concentration of amino acids shown in Table II were in good accordance with data in the literature (Wasserman and Spinelli, 1970; Lee et al., 1974), but it can be concluded that availability of amino acids was reduced through severe processing. Lea and Hannan (1950) reported that free amino acids quickly react with glucose yielding a complex in the ratio of 1:1. The powerful reducing characteristics of the product, as well as the fact that glucose cannot be determined in the acid hydrolysate after a 5-day interaction, indicated that the resulting glucose–amino acid complex was not the same as that in *N*-glycoside. Isomerization of the originally formed *N*-glycoside in isoglucosamine (with the monosubstituted amino group at C_1 and the carbonyl group at C_2) by the Amadori rearrangement explains the stability of the complex to hydrolysis.

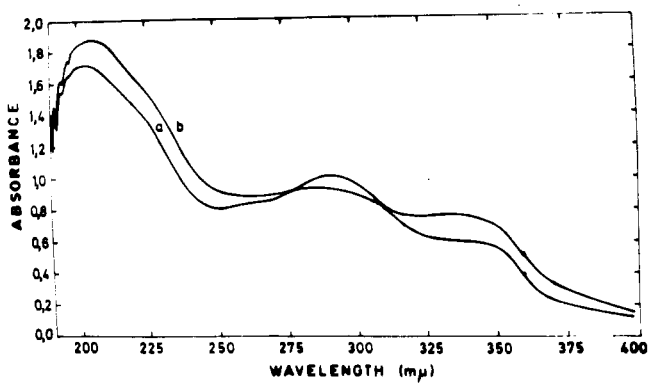

Figure 1. Uv absorption curves of melanoidins: (a) model substance in 60% ethyl alcohol; (b) melanoidin isolated from roasted barley in 60% ethyl alcohol.

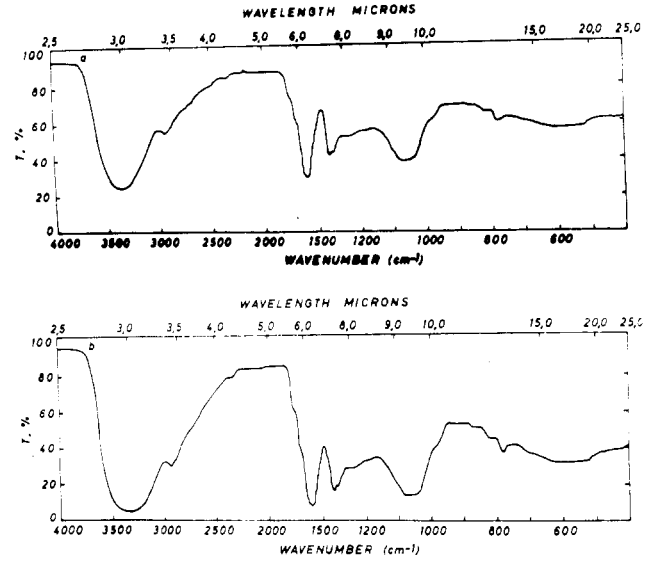

Figure 2. Ir spectra of melanoidins: (a) model substance; (b) melanoidin isolated from roasted barley.

The brown amorphous substance of melanoidins, derived from the reaction between amino acid and glucose, by the cited procedure, showed similar uv and ir spectra as the melanoidin isolated from roasted barley (Figures 1 and 2). Uv absorption curves in the region from 370 to 190 nm of the isolate of brown pigments in roasted barley and the brown amorphous substance of the model system show a rapid increase in absorbance with a decrease in wavelength, especially below 250 nm. Below 275 nm, the curves of uv absorption show slight divergence. Ir spectra of the model substance and of the isolate of the brown pigments from roasted barley are in good agreement. Both spectra have characteristics of carbohydrates, indicated by strong hydroxyl and weak C–H bonds. The presence of a carbonyl group in both cases was not determined. However, absorp-

tion curves indicate the presence of carboxylate ions that could be explained by the presence of Na salts of some organic acids as impurities.

Elementary analyses of the model substance gave the simplest empirical formula of $C_{18}H_{26}O_{11}N$, whereas the empirical formula of the isolate from barley roasted at 250° for 40 min was found to be $C_{18}H_{27}O_{11}N$. Elementary analysis of both the model substance and the isolate from roasted barley indicated that they are of similar composition. The manner by which nitrogen is chemically bound in the brown substance, produced by the process of nonenzymatic browning after the temperature treatment, is not understood. The concentration of the brown substance in roasted barley was unexpectedly high. However, it can be explained by the fact that nonenzymatic browning occurs in two ways. The first occurs from the reaction between amino acid and reducing sugar, and the second occurs from caramelization, which can, but need not, include nitrogen.

Isolation of the brown pigments from roasted barley by means of ion-exchange resin Permutit ES confirms that the substance is of an ionic nature and has a negative charge. According to the results obtained from uv and ir spectra (Figures 1 and 2) and from elementary analysis it was concluded that the isolate of the brown substance from roasted barley was of similar composition to the model substance.

ACKNOWLEDGMENT

The able technological assays of M. Glogovčan are gratefully acknowledged. The authors wish to thank P. Džadžić for his expert assistance.

LITERATURE CITED

Anet, E. F. L. J., *Aust. J. Chem.* **13,** 396 (1960).
Anet, E. F. L. J., *Aust. J. Chem.* **14,** 295 (1961).
Anet, E. F. L. J., *Chem. Ind. (London)* **9,** 262 (1962).
AOAC, "Official Methods of Analysis", 9th ed, Association of Official Agricultural Chemists, Washington, D.C., 1960.
Burton, H. S., McWeeny, D. L., *Chem. Ind. (London)* **11,** 462 (1964).
Grujić-Injac, B., Milić, B. Lj., Piletić, M. V., Lajšić, S., First Conference of Chemists and Technologists of Yugoslavia, Novi Sad, Dec 1971, Paper 16/III.
Holtermand, A., *Die Stärke* **18,** 319 (1966).
Kass, J. P., Palmers, L. S., *Ind. Eng. Chem.* **32,** 1360 (1940).
Knauf, A. E., Hann, R. M., Hudson, C. S., *J. Am. Chem. Soc.* **63,** 1447 (1941).
Lea, C. H., Hannan, R. S., *Nature (London)* **165,** 438 (1950).
Lee, C. M., Chichester, C. O., Lee, T.-C., IVth International Congress of Food Science and Technology, Madrid, Sept 1974, Topic 7a, Paper 11.
Milić, B. Lj., Vlahović, M. N., *J. Food Sci.* **36,** 828 (1971).
Reynolds, T. M., Anet, E. F. L. J., Ingles, D. L., Communication of the 1st International Congress of Food Science and Technology, London, 1962.
Underwood, C. E., Deatherage, F. H., *J. Food Res.* **17,** 419 (1952).
Wasserman, A. E., Spinelli, A. M., *J. Food Sci.* **35,** 328 (1970).
Wiseman, H. G., Mallack, J. C., Jackson, C. O., *J. Agric. Food Chem.* **8,** 78 (1960).
Wolfrom, M. L., *J. Am. Chem. Soc.* **67,** 53 (1945).
Wolfrom, M. L., Plunket, R. A., Liver, M. L., *J. Agric. Food Chem.* **8,** 58 (1960).

Received for review September 19, 1974. Accepted April 23, 1975.

Gas Chromatographic Determination of Thiofanox Residues in Soil, Plants, and Water

Wei-Tsung Chin,* Warren C. Duane, Mary B. Szalkowski, and Don E. Stallard

Thiofanox (P), 3,3-dimethyl-1-(methylthio)-2-butanone *O*-[(methylamino)carbonyl]oxime, undergoes two-step oxidation in soil, plants, and animals to its sulfoxide (P_1), 3,3-dimethyl-1-(methylsulfinyl)-2-butanone *O*-[(methylamino)-carbonyl]oxime, and sulfone (P_2), 3,3-dimethyl-1-(methylsulfonyl)-2-butanone *O*-[(methylamino)-carbonyl]oxime. Procedures are presented for determining the total carbamate residues of the thiofanox (P_t) and some individual metabolites in soil, plants, and water.

Thiofanox (P), 3,3-dimethyl-1-(methylthio)-2-butanone *O*-[(methylamino)carbonyl]oxime, is a potent systemic and contact insecticide developed by Diamond Shamrock Corporation. Metabolic studies in soils (Duane, 1974), plants (Whitten and Bull, 1974), and animals (Sullivan and Tallant, 1974) show that P undergoes rapid oxidation to its sulfoxide (P_1), 3,3-dimethyl-1-(methylsulfinyl)-2-butanone *O*-[(methylamino)carbonyl]oxime, and sulfone (P_2), 3,3-dimethyl-1-(methylsulfonyl)-2-butanone *O*-[(methylamino)carbonyl]oxime. In most cases, only P_2 hydrolyzes to its oxime (O_2), 3,3-dimethyl-1-(methylsulfonyl)-2-butanone oxime. A preferred metabolic pathway of P is outlined in Figure 1 and the LD_{50} values of the residues involved are given in Table I. Judging from the relative toxicities of these six compounds listed in Table I and their quantities determined during metabolic studies, P_2 and P_1 were found to be the major and P and O_2 the minor residues in thiofanox-treated samples. For routine residue analysis, all P and P_1 are first oxidized quantitatively to P_2 which is determined as the total carbamate residues of thiofanox (P_t). This is based on the fact that of these three carbamates, P_2 is the major residue and has the highest toxicity. In this paper, procedures are presented for gas chromatographic determinations of P_t, P, P_1, P_2, and O_2 in soil, potatoes, sugar beets, cottonseeds, cotton gin-trash, foliage, and water.

Diamond Shamrock Corporation, T. R. Evans Research Center, Painesville, Ohio 44077.

EXPERIMENTAL SECTION

Apparatus and Reagents. The instrument used was a Tracor 550 gas chromatograph equipped with a flame photometer detector and a 394-mμ sulfur filter. The chromatographic columns were 6 ft long glass tubing, 0.25 in. o.d. and $^3/_{16}$ in. i.d. Two columns were used: column no. 1 was packed with 1.5% OV-17 and 1.95% OV-210 on Chromosorb W DMCS 60–80 mesh; column no. 2 was packed with 6% DC 200 on 80–100 mesh Gas Chromosorb Q (Tracor, Inc., Austin, Tex.). Florisil (Floridin Co., Pittsburgh, Pa.) was activated at 95° for 10 hr and maintained at this temperature. Cleanup columns were glass tubes, 19 mm i.d. × 400 mm, with a Teflon stopcock. Peracetic acid (FMC, Inorgan-